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The effects of lipoic acid on rat submandibular salivary gland in valproic acid induced oxidative stress

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Valproic acid (VA), an anticonvulsant drug, has been associated with various toxic effects, primarily through the induction of oxidative stress. This study aimed to investigate the potential protective role of alpha lipoic acid (LA), a potent antioxidant, against VA-induced oxidative damage in rat submandibular salivary glands. Control, LA, VA, and VA+LA are groups. LA was given 1 h prior to VA administration. After 16 days VA injection, the rats were decapitated, and submandibular salivary glands were taken, homogenized, and examined by biochemical analyses. Biochemical analyses showed that submandibular salivary gland glutathione (GSH) level, superoxide dismutase (SOD) and glutathione-S-transferase (GST) activities decreased; malondialdehyde (MDA), sialic acid (SA) and nitric oxide (NO) levels, tissue factor activity increased significantly in the VA group compared to the control group. No significant changes were found in catalase and myeloperoxidase activities. In the VA group, LA administration caused significant increases in GSH and NO levels; decreases in MDA, SA levels and SOD, GST activities. These findings suggest that LA may offer a protective effect against VA-induced oxidative damage in the salivary glands, potentially through its antioxidant properties. This study highlights the therapeutic potential of LA in mitigating oxidative stress and tissue damage induced by VA.

Keywords: Alpha lipoic acid, Antioxidant parameters, Oxidative stress, Submandibular salivary gland, Valproic acid

The submandibular gland is the second largest of the three main salivary glands, including the parotid and sublingual glands. The main function of the submandibular glands is to contribute to the production of saliva, which helps to lubricate the oral cavity and aids in the chemical digestion of food. The saliva also coats the food bolus, which makes it easier to swallow. The submandibular gland produces approximately 70% of saliva in the unstimulated state[1]. Drugs for the treatment of various diseases can cause adverse effects such as salivary gland dysfunction, salivary gland hypofunction, sialorrhea (excessive salivation), and dry mouth[2].

Valproic acid (VA) is a branched short-chain fatty acid derived from valeric acid. It is an anticonvulsant drug widely used in the treatment of epilepsy, bipolar disorder, and other neurological disorders[3]. VA is generally well-tolerated and has been proven to be relatively safe, but it has also been found to have many side effects, including oxidative damage in various tissues[3]. Clinical side effects of VA are indigestion, weight gain, dysphoria, fatigue, dizziness, drowsiness, hair loss, headache, nausea, sedation, and tremor. In addition, it has been suggested that VA causes sialadenosis and dry mouth[4], and that VA treatment inhibits the growth of salivary gland tumors[5]. The mechanisms of drug's toxicity are thought to be due to disruption of the oxidant-antioxidant balance, leading to the accumulation of reactive oxygen species (ROS) and oxidative stress. There is evidence demonstrating that VA can modulate oxidative stress in a tissue-specific way[6]. It has not been fully established whether VA can alter oxidative state in major salivary glands. Among the

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tissues affected by VA, the salivary glands, especially the submandibular gland, have been shown to experience changes in markers of oxidative stress.

Alpha lipoic acid (LA) is 1, 2-dithiolane-3-pentanoic acid. LA can be found in plant and animal food sources, such as tomatoes, spinach, broccoli, kidney, liver, and heart. It is also endogenously produced by the liver. It exists in cells as dihydrolipoic acid (DHLA), LA's reduced form. It is essential for mitochondrial aerobic metabolism. The fact that LA is soluble in both fat and water makes it special compared to other antioxidants. This means that it can act in both the plasma membrane and the cytoplasm[7]. LA is a powerful antioxidant, known for its ability to neutralize free radicals and regenerate other antioxidants, such as vitamins C and E. In addition, LA can increase tissue levels of glutathione, an important antioxidant that plays a key role in the detoxification of harmful reactive species in cells[8,9]. Given its antioxidant properties, LA has been proposed as a potential therapeutic agent to alleviate oxidative damage caused by various insults, including drugs like VA. Through its pharmacological effects, such as anticancer, antioxidant, anti-inflammatory, and antiviral effects, numerous studies have reported the effects of LA in improving many diseases[7]. LA ameliorates the hyposalivation of salivary glands induced by therapeutic irradiation. Therefore, it is proposed that LA is a potential agent against radiation-induced hyposalivation in patients with head and neck cancers[10]. However, in another study, LA supplementation did not improve the impaired secretory function of the salivary glands[11].

Considering the key role of oxidative stress in the pathogenesis of salivary gland diseases, this study aimed to investigate the protective effects of LA on oxidative stress and biochemical changes in the submandibular salivary glands of VA-treated rats. Literature relating to the effect on LA on the salivary glands of VA-treated individuals is scarce, and there is evidence demonstrating that VA can induce oxidative stress in a tissue-specific way. Therefore, in this study, key markers of oxidative stress, antioxidant levels, and enzyme activities were measured to specifically assess whether LA administration could alleviate the toxic effects of VA on these glands.

Materials and Methods

Chemicals

The chemicals used in this study were of analytical grade and were obtained from Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA) and Fluka (Buchs, Switzerland) companies. The VA was purchased from Merck (Darmstadt, Germany).

Laboratory animals and experimental design

All experiments in this study were approved by the Marmara University Experimental Animals Ethics Committee (Decision No: 34.2015.mar). Six-months-old Sprague Dawley female rats, weighing 250-350 g, were used. The animals were housed in an animal room which has optimum temperature (20°C ± 2), humidity, and 12 h light/12 h dark conditions. All rats were orally fed pellet-type rat food and fresh tap water. The rats were randomly divided into four groups as follows: Control (C) group (olive oil given group, 1 mL, gavage, n=7); LA given group (50 mg/kg/day, gavage, n=8); VA given group (500 mg/kg/day, intraperitoneal, n=7) and VA+LA given group (in the same doses, n=10). LA was given 1 h prior to VA administration. 16 days after VA injection, the rats were decapitated, and submandibular salivary gland samples were taken and homogenized. Submandibular salivary gland samples were examined by biochemical analyses.

Biochemical examination

The homogenates (10% *w/v*) of submandibular salivary glands were stored in a deep freezer at -80°C until analysis. The homogenates were than centrifuged, and the supernatants were used for the analysis of all biochemical parameters except tissue factor (TF) activity. The homogenate was used directly for TF analysis. Since clotting time is inversely proportional to TF, a lengthening of the clotting time shows decreased TF[12]. The supernatants were used for glutathione (GSH)[13], malondialdehyde (MDA)[14], sialic acid (SA)[15], nitric oxide (NO)[16] levels and superoxide dismutase (SOD)[17], catalase (CAT)[18], glutathione-S-transferase (GST)[19], myeloperoxidase (MPO)[20] activities.

Statistical analyses

Biochemical results were statistically evaluated via GraphPad Prism 9.0. The values were expressed as means ± standard deviation. Since there is a normal distribution, the results were evaluated using an unpaired t-test and analysis of variance (ANOVA), followed by Tukey's multiple comparison tests. Principal component analysis (PCA) was used to visualize the biochemical changes for all exposure conditions. The correlations between the biochemical

parameters were also analyzed with Pearson correlation coefficient. The value of $P< 0.05$ was considered statistically significant.

Results

In the submandibular salivary, the values of the examined biochemical parameters and the significance of the comparisons between groups are shown in (Fig. 1).

In comparison to the control group, the VA-treated rats showed a significant decrease in GSH levels ($P< 0.01$), reflecting impaired antioxidant defenses. This was further supported by decreased activities of SOD ($P< 0.05$) and GST ($P< 0.05$), key enzymes in the oxidative stress response. MDA levels were significantly higher ($P< 0.05$), indicating increased lipid peroxidation and oxidative damage. In addition, SA and NO levels were significantly higher ($P< 0.01$, $P< 0.0001$, respectively), indicating increased oxidative stress and potential inflammation. TF activity was significantly increased ($P< 0.001$), which may indicate a higher clotting potential in response to VA treatment. However, no significant changes were found in CAT ($P> 0.05$) and MPO ($P> 0.05$) activities, which may imply a specific alteration in oxidative stress pathways that do not involve these enzymes.

Compared with the control group, the rats treated with only LA exhibited a significant increase in NO levels ($P< 0.0001$) and TF activities ($P< 0.0001$), a significant decrease in GST ($P< 0.01$) and SOD ($P< 0.01$) activities and in MDA ($P< 0.05$) and SA ($P< 0.05$) levels. But there were no significant changes in CAT ($P> 0.05$) and MPO ($P> 0.05$) activities.

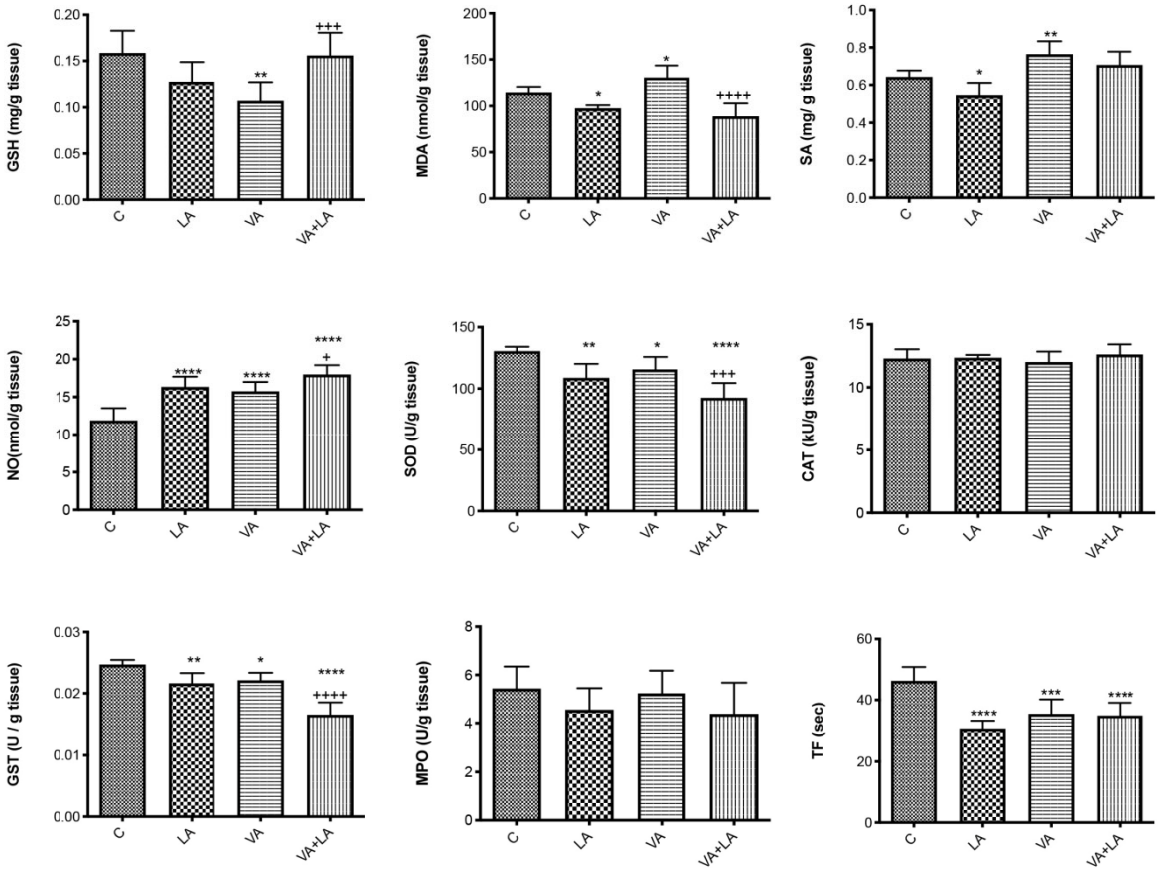

Fig. 1 — The levels of GSH, MDA, SA, NO, and the activities of SOD, CAT, GST, MPO, TF in the submandibular salivary glands in all groups Values are given as mean ± standard deviation. C: Control, LA: Alpha lipoic acid, VA: Valproic acid. GSH: Glutathione, MDA: Malondialdehyde, SA: Sialic acid, NO: Nitric oxide, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-S-transferase, MPO: Myeloperoxidase, TF: Tissue factor, Sec: Second. *$P< 0.05$, **$P< 0.01$, ***$P< 0.001$, ****$P< 0.0001$ significantly different from control. $^{+}P< 0.05$, $^{+++}P< 0.001$, $^{++++}P< 0.0001$ significantly different from VA

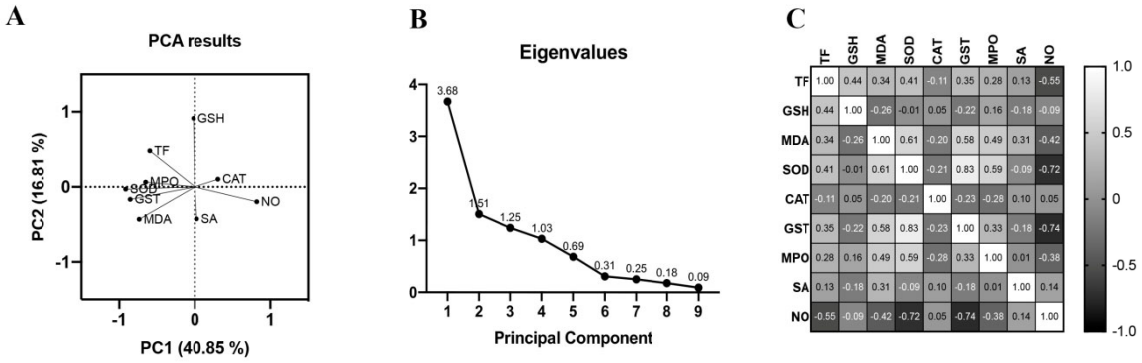

Fig. 2 — Principal component analysis (PCA) of biochemical parameters measured in the submandibular salivary glands of all groups. (A) PCA of biochemical parameters; (B) screen plot representing the eigen values; and (C) and Pearson correlation coefficients between parameters GSH: Glutathione, MDA: Malondialdehyde, SA: Sialic acid, NO: Nitric oxide, SOD: Superoxide dismutase, CAT: Catalase, GST: Glutathione-S-transferase, MPO: Myeloperoxidase, TF: Tissue factor

Compared to the VA group, the LA+VA treated rats showed a significant increase in GSH ($P< 0.001$) and NO ($P< 0.05$) levels, and a significant decrease in MDA levels ($P< 0.0001$). However, GST and SOD activities remained decreased ($P< 0.0001$, $P< 0.001$, respectively). No significant changes were found for TF, CAT, and MPO activities ($P> 0.05$). Overall, the combination of LA and VA partially modulated some of the oxidative stress markers induced by VA in submandibular salivary glands.

The PCA was performed in all groups to explain the effects of LA and VA on the biochemical parameters measured in the submandibular salivary gland (Fig. 2A & B). The correlation coefficients between parameters are seen in Fig.2C. PCA analysis revealed that the first two components detailed around 57.66% of the total variation in the experimental data. PC1 and PC2 explained 40.85% and 16.81% of the total variance, respectively. In first component, CAT, NO, SA data clustered together. These clusters were negatively correlated with GSH, TF, MPO, SOD, GST and MDA.

Discussion

The results of this study provide valuable insights into the protective role of LA against VA-induced oxidative damage in rat submandibular salivary glands. VA is known to cause oxidative stress by disrupting the balance between pro-oxidants and antioxidants, which can lead to cellular damage in various tissues, including salivary glands[21]. The findings of this study reveal significant biochemical changes in the VA-treated group, which were largely reversed by concomitant administration of LA, highlighting the potential of LA as a therapeutic agent in reducing VA-induced oxidative damage.

The effects of VA on submandibular salivary glands' biochemical parameters

Oxidative stress is a common mechanism of drug-induced toxicity, and VA is no exception.VA is commonly used as an antiepileptic and mood stabilizer, and inhibits histone deacetylase. It can prevent salivary gland tumor growth[5], but may also cause side effects like sialadenosis[22], dry mouth[4], oxidative stress, inflammation, and autoimmune reactions[3]. Research on compounds to counteract these effects is important.

The decrease observed in GSH levels and antioxidant enzyme activities such as SOD and GST in this study, which aligns with the results of our previous study[21], can be considered a direct indicator of the drug's effect on the oxidative balance in the tissues. GSH is a critical molecule involved in neutralizing ROS, and its depletion under oxidative stress leads to an inability to combat the damaging effects of ROS and to cellular damage[23]. The decrease in SOD and GST activities further exacerbates this problem, as these enzymes play important roles in scavenging free radicals and detoxifying oxidative by products. SOD and GST are antioxidant enzymes that prevent free radical formation and are also known as primary defense antioxidant enzyme. While SODs convert superoxide radicals to less harmful hydrogen peroxide[24], GST, an enzyme catalyzes the conjugation of electrophiles harmful to cells with GSH, plays a crucial role in protecting against carcinogens, drug toxicities, and various cellular oxidative damage[25]. Consistent with these findings, it has been shown that VA toxicity causes a decrease in GST activities in tissues such as the small intestine[26], pancreas[27] and lens[28].

In this study, the increase in the levels of MDA, a product of lipid peroxidation, and SA, a marker of glycoprotein damage, also reflects oxidative damage to cell membranes and macromolecules. These findings are consistent with our previous study showing that VA causes lipid peroxidation and protein modifications, both of which can compromise cell integrity[21]. The main indicator of VA-induced toxicity is a decrease in GSH levels, coupled with an increase in lipid peroxidation. Experimental studies in other tissues have reported an elevation in MDA levels following VA administration[23,29]. SA, a major component of the secreted mucins, has also been suggested to have antioxidant activity against hydroxyl radicals in mucin in the respiratory and gastrointestinal mucus layers[30]. SA is susceptible to attack by superoxide and related ROS, and it combats oxidative damage caused by H_2O_2. Higher levels of SA in tissues are often associated with increased ROS levels[31].

In the present study, the increased NO levels observed in the VA group further promote an inflammatory response, as NO is typically released during inflammatory processes and may contribute to further oxidative damage. In addition to inhibiting lipid oxidation, NO can directly neutralize ROS by its prooxidant and antioxidant properties[32]. This increase may be attributed to inflammation and inflammatory responses triggered by the accumulation of ROS and toxic VA metabolites, potentially due to the overexpression of NOS or activation of the NO-citrulline cycle[33].

CAT is an important antioxidant enzyme responsible for converting hydrogen peroxide to water and oxygen[34], while MPO, is an enzyme, produced by neutrophils during inflammation[35]. No significant changes were observed in CAT and MPO activities in the VA group. The lack of change in MPO also suggests that although VA may trigger some level of inflammatory response, the effects may not be strong enough to activate MPO significantly in the submandibular glands. This may indicate that the primary mode of damage in this model is oxidative rather than inflammatory, or that the inflammatory response does not progress to the point where MPO activity is significantly elevated.

It has been suggested that elevated levels of ROS may induce TF gene expression. VA is a cause of hematological problems, and coagulation abnormalities are frequently observed during VPA treatment[36]. The abnormal expression of TF is associated with thrombotic complications in a variety of diseases, including atherosclerosis, cancer, and inflammation. The increase in TF activity in VA-treated rats suggests an elevated pro-inflammatory and pro-coagulant state. TF is a key initiator of the coagulation cascade, and its upregulation is often associated with inflammation and cellular damage[37]. It has been shown that VA increased TF activity in gingival tissue[38]. In parallel with the findings of previous studies, these changes suggest that VA not only disrupts the oxidative balance but also triggers inflammatory pathways, contributing to overall tissue damage in the submandibular salivary gland.

The PCA results for the biochemical parameters measured in the submandibular salivary gland suggest a meaningful structure in the data, highlighting key relationships among the variables. The first two principal components (PC1 and PC2) explained a substantial portion of the total variation in the dataset (57.66%), with PC1 alone accounting for 40.85% (Fig. 2). This indicates that the first two components capture a significant amount of the variation in the submandibular salivary glands biochemical profile.

The clustering of parameters such as CAT, NO, and SA in PC1 may reflect these observed changes in the VA group, where higher NO and SA levels may be associated with increased oxidative stress, as indicated by the increase in MDA, while antioxidant defenses (GSH, SOD, GST) were reduced. These findings could imply that higher enzymatic or metabolic activity, associated with NO, and SA, might be linked to a compensatory reduction in oxidative stress markers. The separation of these parameters into distinct clusters within the PCA plot provides an insightful view into the biochemical relationships at play in the submandibular salivary gland. Further investigation into these clusters could help clarify the underlying physiological processes and how they are interrelated in response to certain conditions or stimuli. Additionally, the second principal component (PC2), while explaining a smaller portion of the variation (16.81%), might capture secondary biological distinctions or interactions between the parameters not fully accounted for in PC1.The increase in TF activity in the submandibular salivary gland caused by VA also supports the view that VA not only disrupts the oxidative balance but also triggers inflammation.

The effects of LA on submandibular salivary glands' biochemical parameters

LA is a powerful antioxidant known for its ability to scavenge free radicals and regenerate other antioxidants such as vitamins C and E[8,9]. In this study, LA administration provided significant protection against VA-induced oxidative damage. The observed significant increase in GSH levels after LA treatment suggests that LA may help replenish cellular stores of this critical antioxidant, provide better detoxification of ROS, and reduce oxidative stress. It has been proposed that LA increases GSH levels by enhancing the availability of cysteine[39].

However, SOD and GST activities were more decreased by LA in both the LA and VA+LA groups compared to the respective groups (C and VA). These results suggested that H_2O_2 degradation processes were unchanged and may have increased accumulation of superoxide anion and the formation of hydroxyl radicals. In the literature, it has been shown that LA induces apoptosis by increasing mitochondrial superoxide anion production in cancer cells[40]. However, further research is needed to explain the mechanisms behind these changes in the submandibular salivary glands.

The decrease in MDA and SA levels in the LA-treated groups, compared to the respectives, is particularly notable. These markers are indicative of lipid peroxidation and glycoprotein oxidation, both of which contribute to cellular dysfunction[41]. Higher levels of SA have also been linked with inflammation[42]. By reducing these markers, LA appears to preserve the structural integrity of submandibular salivary gland cells, and prevent damage to cell membranes and macromolecules.

NO, a product of normal endothelium, is a principal determinant of normal endothelial and vascular function[43]. During inflammation, NO production by the vasculature increases considerably and, in conjunction with other ROS, contributes to oxidative stress. The increased NO levels in the LA-treated groups may indicate a beneficial role in maintaining vascular function or in the regulation of oxidative stress. Although NO is often associated with inflammation[44], its increased levels in this context may be indicative of the ability of LA to regulate oxidative pathways and endothelial function, potentially contributing to tissue protection. NO reacts with ROS to produce peroxynitrite and can alter protein and lipid structure. NO has important neurotransmitter and regulatory roles when released at low levels. However, when combined with superoxide to form harmful peroxynitrite, high levels of NO (produced by the increased activity of inducible NO synthase during inflammation) have a negative effect[44]. LA also exhibited a significant increase in TF activities. This may be linked with inflammation. Interestingly, no significant change was found in CAT and MPO activities with LA administration in the study. The lack of significant changes in these enzymes may suggest that the protective effects of LA are not mediated through h these specific antioxidant pathways. It is possible that LA acts through alternative mechanisms, such as its ability to regenerate GSH or to directly scavenge free radicals. Alternatively, the lack of response may be related to specific conditions of the study, such as the dose or duration of LA administration, which may not have been sufficient to induce significant changes in CAT and MPO activities.

These results collectively indicate that while LA treatment, both alone and in combination with VA, can modulate certain oxidative stress markers, it does not fully reverse the alterations in antioxidant enzyme activities induced by VA. The PCA analysis supports these findings, demonstrating how the biochemical changes are reflected in the data and providing further insight into the complex interplay between these treatments in modulating oxidative stress and antioxidant defense mechanisms in the salivary glands.

The limitation of this study is that the long-term effects of LA treatment were not examined. Chronic LA administration may yield different results compared to short-term interventions. Future research should include investigating the molecular mechanisms by which LA regulates antioxidant pathways and inflammation, as this may help elucidate the full scope of its protective effects.

Conclusion

This study provides evidence that short-term treatment LA can mitigate the oxidative stress induced by VA in the rat submandibular salivary glands. Improvements in antioxidant levels and reductions in oxidative damage markers support this finding. LA's ability to restore GSH levels, enhance antioxidant enzyme activity, and reduce lipid peroxidation highlights its potential as a therapeutic agent for alleviating drug-induced oxidative damage.

Conflict of interests

All authors declare no conflict of interest.

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